

Ghrelin Regulates Insulin Release and Glycemia: Physiological Role and Therapeutic Potential

Toshihiko Yada^{1,*}, Katsuya Dezaki¹, Hideyuki Sone^{1,2}, Masaru Koizumi^{1,3}, Boldbaatar Damdindorj¹, Masanori Nakata¹ and Masafumi Kakei⁴

¹Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Yakushiji 3311-1, Shimotsuke, Tochigi 329-0498, Japan; ²Department of Human Life and Environmental Science, Niigata Women's College, Ebigase, Niigata 950-8680, Japan; ³Department of Surgery, Jichi Medical University School of Medicine, Yakushiji 3311-1, Shimotsuke, Tochigi 329-0498, Japan; ⁴Department of Comprehensive Medicine, Saitama Medical Center, Jichi Medical University, Saitama 337-0043, Japan

Abstract: Insulin release from pancreatic islet β -cells is stimulated by glucose. Glucose-induced insulin release is potentiated or suppressed by hormones and neural substances. Ghrelin, a novel acylated 28-amino acid peptide isolated from stomach, is the endogenous ligand for the growth hormone (GH) secretagogue-receptor (GHS-R). Circulating ghrelin is produced predominantly in stomach. Ghrelin is a potent stimulator of GH release and feeding as well as exhibiting positive cardiovascular effects. In relation to the glucose metabolism, initial studies indicated that low plasma ghrelin levels are associated with elevated fasting insulin levels, insulin resistance, and obesity. It has recently been demonstrated that ghrelin suppresses glucose-induced insulin release via $G\alpha_{12}$ subtype of GTP-binding proteins and delayed outward K^+ (Kv) channels, representing a novel signaling mechanism, and that the ghrelin originating from islets regulates insulin release and thereby glycemia. Furthermore, elimination of ghrelin enhances insulin release to prevent or ameliorate glucose intolerance in high-fat diet fed mice and ob/ob mice. This review focuses on the physiological roles of ghrelin in regulating insulin release and glycemia, the insulinostatic mechanisms of ghrelin in islet β -cells, and the potential of ghrelin-GHS-R system as the therapeutic target to treat type 2 diabetes.

Keywords: Ghrelin, GHS-R, Insulin release, Islet β -cell, Kv channel, Diabetes, Ghrelin-knockout.

INTRODUCTION

Ghrelin, a novel acylated 28-amino acid peptide, was isolated from human and rat stomach as the endogenous ligand [1] for the growth hormone (GH) secretagogue-receptor (GHS-R) [2]. Circulating ghrelin is produced predominantly in stomach [3]. Ghrelin is a potent stimulator of GH release [1] and feeding [4] as well as exhibiting positive cardiovascular effects [5]. In addition, the plasma ghrelin level correlates inversely with obesity [6-8]. Low plasma ghrelin levels are associated with elevated fasting insulin levels and insulin resistance [9, 10].

Systemic administration of ghrelin increases blood glucose [11-14] and decreases plasma insulin levels [12,14] in fasted states and glucose tolerance tests (GTT) in humans and rodents. Ghrelin inhibits glucose-induced insulin release in perfused pancreas and isolated islets. In rat single β -cells, ghrelin inhibits glucose-induced increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and activates delayed outward K^+ (Kv) channels. Ghrelin stimulates $G\alpha_{12}$ subtype of GTP proteins to activate Kv, thereby suppressing glucose-induced $[Ca^{2+}]_i$ increases and insulin release in islet β -cells [15].

GHS-R antagonists increase plasma insulin and decreased glycemia, showing a systemic role of endogenous ghrelin. Ghrelin is expressed in pancreatic islet cells. GHS-R antagonism, ghrelin immunoneutralization and ghrelin-knockout (Ghr-KO) mice display enhanced glucose-induced insulin release from perfused pancreas and isolated islets [14, 16]. Thus, pharmacological, immunological and genetic blockades of ghrelin in pancreatic islets all markedly augment glucose-induced insulin release, demonstrating that islet-derived ghrelin is a physiologic attenuator of insulin release in rodents.

High-fat diet (HFD)-induced glucose intolerance is prevented in Ghr-KO mice due to markedly enhanced insulin release [16]. Ghr-KO also ameliorates diabetic phenotype of ob/ob mice by promoting insulin release [17]. Thus, elimination of ghrelin enhances insulin release to ameliorate glucose intolerance associated with obesity. Manipulation of insulinostatic function of ghrelin-GHS-R system in

islets could optimize the amount of insulin release to meet the systemic demand, providing a potential therapeutic application to prevent type 2 diabetes.

1. SYSTEMIC EFFECTS OF GHRELIN: EXOGENOUS GHRELIN ELEVATES PLASMA GLUCOSE AND ATTENUATES INSULIN LEVELS

Systemic action of exogenous ghrelin to elevate blood glucose levels has been well documented in humans and rodents [11-14]. In mice fasted overnight, intraperitoneal (i.p.) administration of ghrelin at concentrations of 1 and 10 nmol/kg significantly elevated blood glucose levels at 30 min after administration [14]. The hyperglycemic effect of ghrelin was completely blocked by simultaneous administration of GHS-R antagonist, [D-Lys³]-GHRP-6. Des-acyl ghrelin, a form of ghrelin that is incapable of activating GHS-R [1, 18], failed to significantly alter blood glucose levels [14]. These results indicate that ghrelin increases blood glucose via specific interaction with GHS-R. The ghrelin-induced hyperglycemia appears to involve neither GH, a hyperglycemic hormone, nor insulin resistance, based on the following observation. Firstly, ghrelin increased blood glucose in GH-deficient *little* mice and control wild mice in a similar manner. Secondly, in insulin tolerance test (ITT), i.p. injection of insulin lowered blood glucose levels, in the ghrelin-administered and control mice in a similar manner. By contrast, when ghrelin at 1 and 10 nmol/kg was simultaneously injected with glucose in GTT, the insulin responses were markedly attenuated and the glucose responses were larger in comparison to control. These results indicate that the hyperglycemic effect of ghrelin is neither due to the ability of ghrelin to release GH nor to induction of insulin resistance, but primarily caused by reduction of plasma insulin levels.

2. IN VITRO EFFECTS OF GHRELIN: EXOGENOUS GHRELIN INHIBITS GLUCOSE-INDUCED INSULIN RELEASE IN PANCREAS AND ISLETS BY ACTIVATING KV CHANNELS AND SUPPRESSING Ca^{2+} SIGNALING IN β -CELLS

2-1. Ghrelin Suppresses Insulin Release in Perfused Pancreas and Isolated Islets and Attenuates $[Ca^{2+}]_i$ in Single β -Cells

Elevation of glucose concentration from 2.8 mM to 8.3 mM stimulated insulin release from perfused pancreas in a biphasic man-

*Address correspondence to this author at the Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Yakushiji 3311-1, Shimotsuke, Tochigi 329-0498, Japan; Tel: +81-285-58-7320; Fax: +81-285-44-9962; E-mail: tyada@jichi.ac.jp

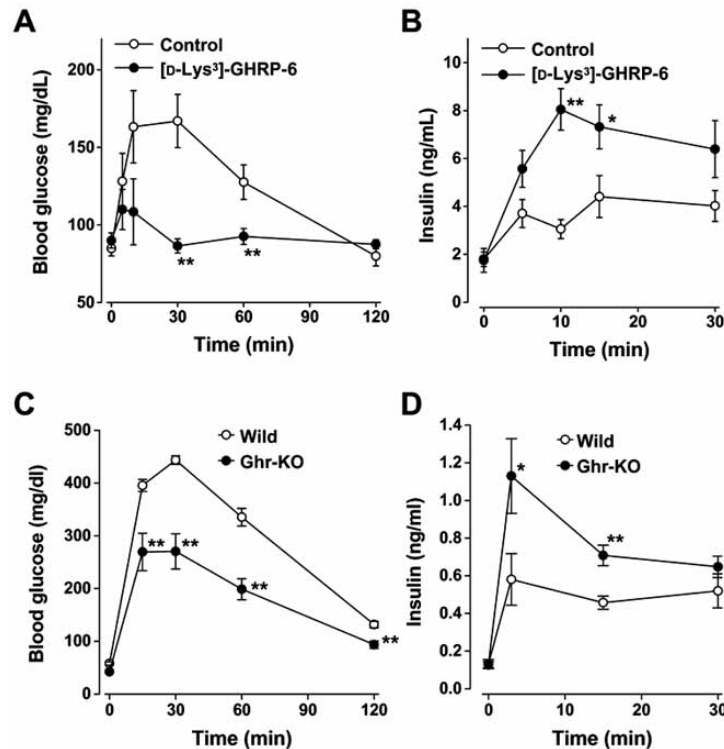


Fig. (1). Both pharmacologic blockade and gene-knockout of ghrelin attenuate plasma glucose and increase insulin responses in glucose tolerance test (GTT). (A and B) GHS-R antagonist, [D-Lys³]-GHRP-6 (10 μ mol/kg i.p.), decreased blood glucose levels and increased insulin levels during GTT (glucose 1 g/kg i.p.) in ddY mice ($n = 6-10$). * $P < 0.05$; ** $P < 0.01$ vs. control. (C and D) In GTT (glucose 2 g/kg i.p.), male Ghr-KO mice exhibited attenuated elevations of blood glucose and enhanced elevations of insulin levels in comparison to wild-type mice ($n = 9-10$). * $P < 0.05$; ** $P < 0.01$ vs. wild-type mice. "Copyright © 2004 American Diabetes Association From Diabetes[®], 2004; Vol. 53, 3142-3151 and © 2006 American Diabetes Association From Diabetes[®], 2006; Vol. 55, 3486-3493. Reprinted with permission from *The American Diabetes Association*."

ner. Both the first and second phases of glucose-induced insulin release were suppressed by administration of exogenous ghrelin at a relatively high concentration of 10 nM [14]. In isolated islets, glucose-induced insulin release was attenuated by ghrelin at 10 nM. In contrast, basal insulin release at 2.8 mM glucose in perfused pancreas and isolated islets were not affected by ghrelin. Ghrelin at 10 nM markedly suppressed the peaks of the first phase $[Ca^{2+}]_i$ increases and the second phase $[Ca^{2+}]_i$ oscillations in response to glucose [14]. The attenuation of $[Ca^{2+}]_i$ responses by ghrelin was abolished in the presence of GHS-R antagonist.

2-2. Ghrelin Activates Delayed Outward K⁺ (K_v) Currents in Single β -Cells

In patch clamp study of rat single β -cells under a perforated whole-cell clamp mode, ghrelin increased the amplitudes of K⁺ currents in a reversible manner [14]. This response occurred in the presence of tolbutamide, a blocker of ATP-sensitive K⁺ (K_{ATP}) channel. Current-voltage relations of this current depicted that ghrelin activated the delayed outward K⁺ currents (K_v) at the potentials positive to -30 mV. This enhancing effect was blunted when 10 mM tetraethylammonium (TEA), a blocker of delayed-rectifier K⁺ channels, was administered during exposure to ghrelin. Furthermore, in the presence of TEA, the ability of ghrelin to inhibit glucose-induced $[Ca^{2+}]_i$ increase [14] and insulin release [15] were both partially but significantly diminished. Moreover, stromatoxin (0.1 μ M), a specific blocker of the 2.1 subtype of K_v channels [19], significantly diminished the ghrelin stimulation of K⁺ currents [15]. Furthermore, stromatoxin potentiated glucose-induced insulin release, and in the presence of stromatoxin ghrelin failed to attenuate glucose-induced insulin release [15]. These results suggest that the ability of ghrelin to inhibit $[Ca^{2+}]_i$ increases and insulin release is partly due to the en-

hancement of the TEA-sensitive K_v current, most likely passing through the stromatoxin-sensitive K_v2.1 channels.

2-3. Ghrelin Acts via G α_{i2} in β -Cells

In the rats treated with pertussis toxin (PTX), an agent that inhibits Gi/Go subtypes of GTP-binding proteins, i.p. ghrelin injection failed to decrease plasma insulin levels [15]. The *in vitro* effects of ghrelin on insulin release, $[Ca^{2+}]_i$ and K_v channels were all blunted in islets and β -cells treated with PTX [15]. Furthermore, ghrelin failed to suppress $[Ca^{2+}]_i$ increase and insulin release in β -cells treated with antisense oligonucleotide specific for G-protein G α_{i2} subunit, whereas antisense oligonucleotides against G α_{i1} and G α_{i3} were ineffective [15].

These results indicate that ghrelin inhibits insulin release primarily via PTX-sensitive G α_{i2} -mediated inhibition of $[Ca^{2+}]_i$ increases, in which enhancement of TEA- and stromatoxin-sensitive K_v2.1 channel plays a pivotal role. This is a novel signaling pathway for ghrelin-GHS-R system that is unique to islet β -cells.

3. PHYSIOLOGICAL ROLE OF GHRELIN: ENDOGENOUS GHRELIN REGULATES SYSTEMIC INSULIN AND GLUCOSE LEVELS

3-1. GHS-R Antagonists Increase Plasma Insulin and Decrease Glucose Levels

In fasted mice, i.p. administration of GHS-R antagonists, [D-Lys³]-GHRP-6 and [D-Arg¹, D-Phe⁵, D-Trp^{7,9}, Leu¹¹]-substance P (SPA) [20], reduced fasting blood glucose concentrations at 30 and 60 min in a dose-dependent manner [14]. In GTT, increases in plasma glucose were markedly attenuated by simultaneous injection of [D-Lys³]-GHRP-6 (Fig. 1A), while increases in plasma insulin were markedly enhanced by the GHS-R antagonist (Fig. 1B).

3-2. Ghrelin-KO Mice Display Increased Plasma Insulin and Decreased Blood Glucose Levels

Acylated-ghrelin was undetectable in the plasma of ghrelin knockout (Ghr-KO) mice [16]. When fed standard chow, male Ghr-KO and wild-type (C57BL/6J) mice at 8 weeks of age exhibited no significant differences in body weights, total 24-hr food intake, and blood glucose levels in fed states. The results are consistent with those in other lines of Ghr-KO mice [21-24]. In GTT, in contrast, Ghr-KO mice exhibited markedly enhanced insulin responses and attenuated glucose responses (Fig. 1C and D).

3-3. Blood Glucose-Lowering Effect Largely Results from Increased Plasma Insulin

In ITT, the time course and magnitude of the changes of blood glucose levels following i.p. insulin injection were indistinguishable between the GHS-R antagonist-injected and control mice [14]. Furthermore, Ghr-KO and wild-type mice exhibited similar profiles of ITT [16]. These results suggest that endogenous ghrelin does not significantly alter insulin sensitivity. Hence, the alteration of glycaemic responses to GTT with ghrelin receptor antagonist and in Ghr-KO mice result primarily from enhanced insulin secretion, though possible additional effects of ghrelin on glucose production [25] or insulin sensitivity [26] cannot be disregarded. These findings indicate a physiological function of endogenous ghrelin to lower plasma insulin and thereby upwardly control glycemia.

4. PANCREATIC ISLET-DERIVED GHRELIN SERVES AS A PHYSIOLOGICAL REGULATOR OF INSULIN SECRETION

4-1. Plasma Insulin Level is Downregulated by Endogenous Ghrelin in Gastrectomized Rats

It has been shown that as large as 70% of the circulating ghrelin originates from stomach, while the rest is derived from other tissues, including intestine and pancreas. The insulinostatic function of endogenous ghrelin could be operated by the ghrelin derived from stomach and/or that derived from other tissues. This issue was addressed by employing gastrectomized (GX) rats lacking stomach-derived ghrelin. In GX rats, the plasma concentration of acylated-ghrelin was reduced to a level less than 20% of control [16], reflecting the lack of stomach-derived ghrelin. I.p. injection of GHS-R antagonist [D-Lys³]-GHRP-6 in GX rats markedly increased plasma insulin concentrations and the increment was as large as that observed in normal rats [16]. Thus, the increase in plasma insulin due to blockade of ghrelin receptor was altered neither by the absence of stomach nor by dramatically reduced level of circulating ghrelin. The result indicates that the insulinostatic function of endogenous ghrelin that is counteracted by GHS-R antagonist is not executed by stomach-derived ghrelin but primarily by non-circulating ghrelin that is produced locally. This finding suggests that the ghrelin produced by pancreas could serve as a local regulator of insulin release, although it may not contribute to the level of circulating ghrelin.

4-2. Expressions of Ghrelin and GHS-R in Pancreatic Islets

Ghrelin mRNAs were expressed in the pancreas and isolated rat islets [27]. Immunohistochemistry with antiserum against ghrelin (1-11) demonstrated the immunoreactivity for acylated-ghrelin (active ghrelin) in a fraction of islet cells [14, 27]. Acylated-ghrelin was detected in islets using RIA using antiserum against ghrelin (1-11) [14]. Furthermore, immunofluorescence double staining revealed that ghrelin-immunoreactive cells were observed mainly in the periphery of rat islets where glucagon-immunoreactive cells were located [14, 27].

Ghrelin-immunoreactivity has also been demonstrated in islet β -cells [28] and islet ghrelin cells [29-31] including those named ϵ -cells [32]. In immature islet cells of rats, ghrelin is expressed together with

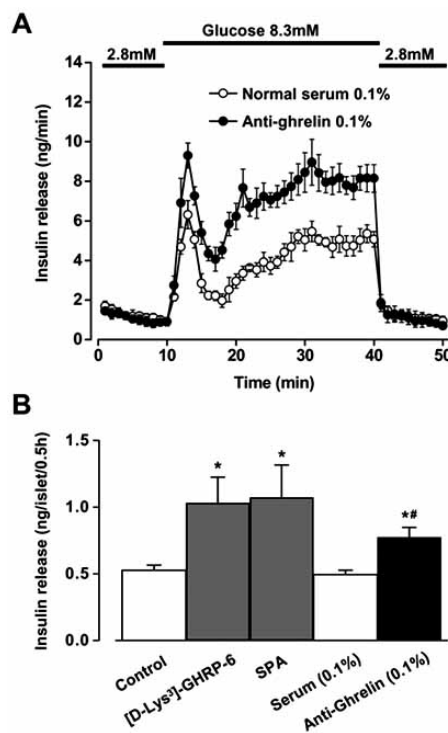


Fig. (2). Immunoneutralization and pharmacological blockade of ghrelin increase insulin secretion in perfused rat pancreas and in rat islets. (A) Immunoneutralization of endogenous ghrelin using antiserum against ghrelin (1-11) (0.1%) enhanced glucose (8.3 mM)-induced insulin release in perfused rat pancreas ($n = 3-4$). (B) Insulin release at 5.6 mM glucose was enhanced by GHS-R antagonist, [D-Lys³]-GHRP-6 (1 μ M) and SPA (1 μ M), and by antiserum against ghrelin (1-11) (0.1%), but not nonimmune serum (0.1%), in rat islets. Data represent the means \pm s.e.m. of 10 experiments ($n = 10$). "Copyright © 2004 American Diabetes Association From Diabetes[®], 2004; Vol. 53, 3142-3151 and © 2006 American Diabetes Association From Diabetes[®], 2006; Vol. 55, 3486-3493. Reprinted with permission from *The American Diabetes Association in slightly modified form.*"

glucagon or pancreatic polypeptide [30]. Thus, ghrelin could be expressed by multiple islet cell types, depending on specific conditions and ages of animals/humans. On the other hand, GHS-R mRNA and protein are expressed in the pancreas of rats and humans [1, 27, 28] and in β -cell lines [30].

4-3. Release of Ghrelin from Pancreas

Release of ghrelin from pancreatic islets was assessed by comparing the ghrelin level in the pancreatic vein (splenic vein) with that in the pancreatic artery (celiac artery) in anaesthetized rats. The concentrations of both acylated-ghrelin and desacyl-ghrelin in the pancreatic vein were significantly higher (approximately 8 times and 3 times, respectively) than those in the pancreatic artery, suggesting that ghrelin is released from pancreas [16].

4-4. GHS-R Blockade and Ghrelin Immunoneutralization Enhance Insulin Release in Perfused Pancreas

Physiological role of the pancreatic islet-produced ghrelin was addressed by studying insulin release from the perfused rat pancreas, an *in vitro* system that well retains the intact circulation in pancreatic islets and excludes the influence of other organs [16]. A rise in the perfusate glucose concentration from 2.8 to 8.3 mM evoked insulin release in a biphasic manner (Fig. 2A). Both the first and second phases of glucose-induced insulin release were significantly enhanced by immunoneutralization of endogenous ghrelin with anti-ghrelin antiserum (Fig. 2A) and by blockade of GHS-R [16].

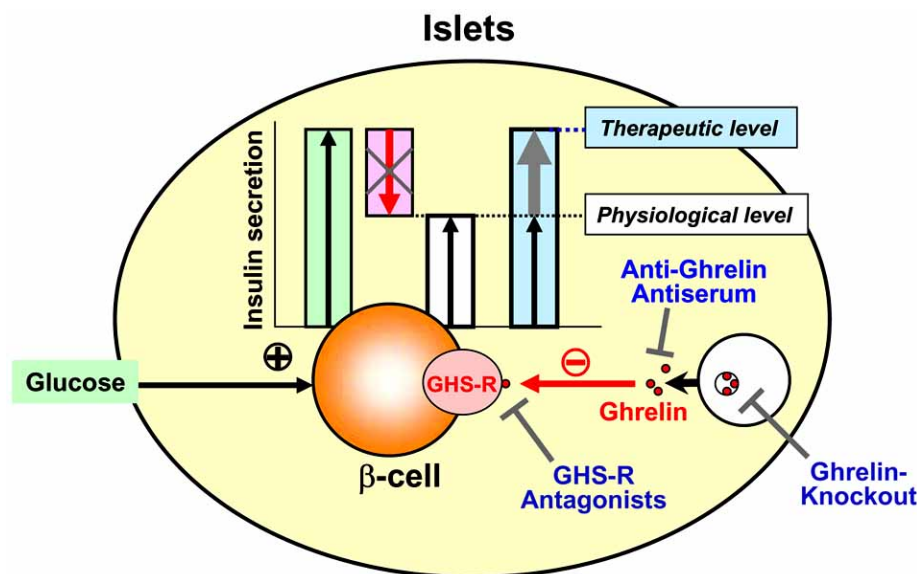


Fig. (3). Model for paracrine/autocrine role of ghrelin in islets. Ghrelin is produced and released from pancreatic islet cells and act on islet β -cells to suppress glucose-induced insulin release, determining the physiological level of insulin release. Pharmacological, immunological and genetic blockades of ghrelin in pancreatic islets enhance glucose-induced insulin release, achieving therapeutic levels of insulin release.

4-5. GHS-R Antagonists and Anti-Ghrelin Antiserum Enhance Insulin Release and $[Ca^{2+}]_i$ Increase in Islets

In isolated rat islets, both anti-ghrelin antiserum and GHS-R antagonists, [D-Lys³]-GHRP-6 and SPA, markedly increased insulin release (Fig. 2B) [14]. Furthermore, the peaks of the first phase $[Ca^{2+}]_i$ increases and the second phase $[Ca^{2+}]_i$ oscillations in response to glucose, measured in whole islets, were enhanced by the presence of [D-Lys³]-GHRP-6 [14]. Similarly, anti-ghrelin antiserum enhanced glucose-induced $[Ca^{2+}]_i$ increases in whole islets.

4-6. Ghrelin-KO Mice Display Increased Insulin Release in Isolated Islets

The number (density) and average size of islets were not significantly different between wild-type and Ghr-KO mice. Glucose-induced insulin release from isolated islets of Ghr-KO mice was significantly greater than that of wild-type mice, while basal levels of insulin release were not altered [16]. No difference was observed between Ghr-KO and wild-type mice in insulin content per islet and mRNA expressions of insulin 1 and insulin 2. These data indicate that the larger amount of insulin release in islets of Ghr-KO mice results from greater insulin secretory response to glucose.

The finding that pharmacological, immunological and genetic blockades of ghrelin in pancreatic islets enhance glucose-induced insulin release suggests that ghrelin is released from pancreatic islet cells and act on islet β -cells to suppress glucose-induced insulin release (Fig. 3).

5. GHRELIN-GHS-R SYSTEM AS A THERAPEUTIC TARGET FOR TYPE 2 DIABETES

5-1. Ghrelin-KO Counteracts Glucose Intolerance in High-Fat Diet-Fed and ob/ob Mice

When wild-type and Ghr-KO mice were fed high-fat diet (HFD) for 4 weeks, they displayed moderate increases in body weight to a similar extent. HFD resulted in significant increases in blood glucose levels in wild-type mice but not in Ghr-KO mice [16]. HFD also increased plasma insulin levels, and this change was much greater in Ghr-KO than wild-type mice. Thus, Ghr-KO mice displayed a phenotype of enhanced insulin release and nearly normal glycemia under HFD conditions. This phenotype was even more prominent in GTT. In wild-type mice, increases in blood glucose levels were exaggerated

in HFD group compared to control diet group, exhibiting HFD-induced glucose intolerance (Fig. 4A). Although insulin response to GTT also tended to be enhanced in HFD group, the change was not statistically significant (Fig. 4B). In Ghr-KO mice, by contrast, increases in blood glucose levels in HFD group were not significantly different from those of control diet group, and insulin response was markedly enhanced in HFD group (Fig. 4C and D). Thus, ghrelin-deficiency promoted insulin release and prevented glucose intolerance in a HFD-induced obese model (Fig. 3).

Sun Y *et al.* [17] have recently reported that in ob/ob mice, a genetic model of obesity due to leptin-deficiency, ablation of ghrelin augmented insulin release and thereby markedly reduced hyperglycemia. Thus, the ghrelin blockade counteracts the obesity-associated glucose intolerance in two different obese models. As the underlying mechanism, we propose that lack of ghrelin and its insulinostatic activity may increase the maximal capacity of glucose-induced insulin release and enable islets to secrete more insulin to meet an increased demand associated with obesity, thereby achieving normoglycemia (Fig. 3).

5-2. Chronic Effects of Ghrelin System and its Inhibition on the Metabolism

It was shown that administration of ghrelin increases IA-2 β mRNA in mouse pancreas and that inhibition of IA-2 β expression by the RNA interference technique ameliorates ghrelin's inhibitory effects on glucose-stimulated insulin secretion in MIN6 insulinoma cells [33]. In ob/ob mice, ablation of ghrelin reduces the expression of uncoupling protein 2 (UCP2) [17], a mitochondrial protein that negatively regulates glucose-induced ATP production and thereby insulin release in β -cells [34]. Therefore, it is likely that ghrelin inhibits insulin release via two modes of action in β -cells: it acutely activates Kv channels and suppresses Ca^{2+} signaling, while chronically it may also upregulate UCP2 and IA-2 β . In ob/ob mice, the increment of insulin release due to ghrelin-KO is remarkably large [17], which could be due at least partly to upregulation of UCP2 in islet β -cells [34], as well as in the hypothalamic feeding center [35], of this animal model. Moreover, the enhanced ghrelin action in ob/ob mice, assessed by the effect of ghrelin-deficiency, could be due to the lack of leptin, since leptin and ghrelin are considered mutual antagonists. To support it, leptin counteracts the effects of ghrelin in several systems including the regulation of feeding and the neuropeptide Y neuron activity in

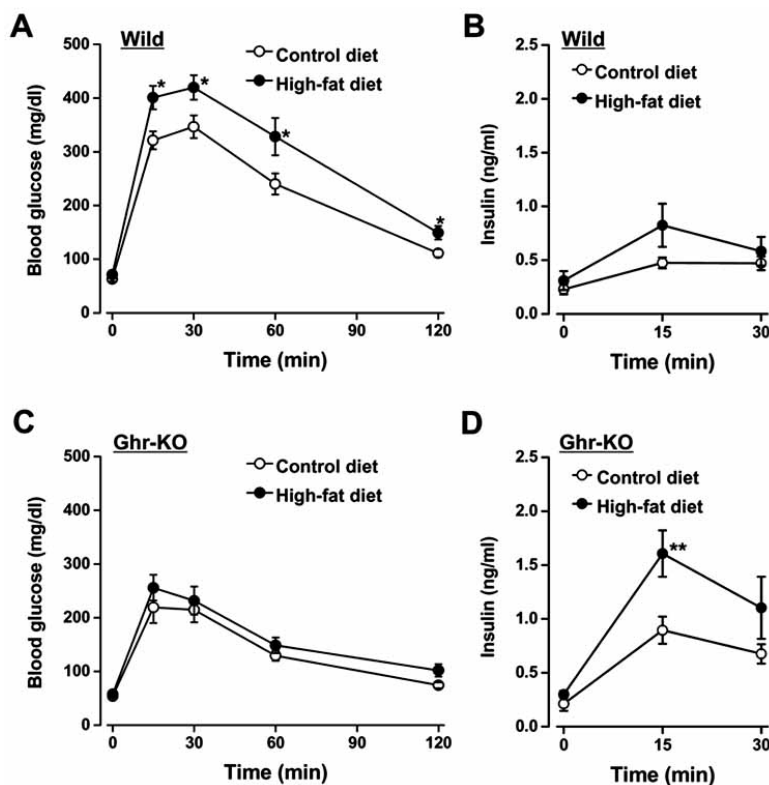


Fig. (4). High-fat diet-induced glucose intolerance is prevented in ghrelin knockout mice. The mice were given HFD or control diet from 8 to 12 weeks old. In wild-type mice, HFD group exhibited glucose intolerance (A) and slight enhancement of insulin release (B) during GTT (glucose 2 g/kg i.p.). In Ghr-KO mice, glycemic responses to GTT in HFD group were not different from those of control diet group (C), and insulin response at 15 min was markedly enhanced in HFD group (D). * $P < 0.05$; ** $P < 0.01$ vs. control diet mice. "Copyright © 2006 American Diabetes Association From *Diabetes*, 2006; Vol. 55, 3486-3493. Reprinted with permission from *The American Diabetes Association*."

the hypothalamic arcuate nucleus [36]. In this context, it is worth noting that ghrelin, contrary to leptin, has several actions that could promote metabolic syndrome; it inhibits insulin release and elevates glycemia, stimulates feeding, and increases adiposity [37]. Therefore, suppression of these ghrelin actions has a potential to counteract diabetes, hyperphagia and obesity simultaneously, thereby acting against metabolic syndrome. However, it should be kept in mind that ghrelin also stimulates release of growth hormone [1], a factor that decreases the fat mass and increases the muscle mass. The interplay between ghrelin and adipocytokines, such as leptin and adiponectin, and the impact of suppression of ghrelin-GHS-R system in the regulation of metabolism remain to be further elucidated.

CONCLUSION

Both pharmacological blockade and genetic deletion (Ghr-KO) of ghrelin enhanced insulin responses and reduced glucose responses in GTT. The finding reinforces the concept that endogenous ghrelin serves as a physiological regulator of systemic insulin and glycemia. Under conditions in which the systemic demand for insulin exceeds the physiological range, such as early stages of diet-induced obesity and/or insulin resistance, antagonizing ghrelin function can promote insulin secretion and prevent glucose intolerance, providing a potential therapeutic avenue to counteract the progression of type 2 diabetes (Fig. 3).

The notion that the islet-derived ghrelin plays a central role in the regulation of insulin release is supported by the following findings. (1) Systemic administration of GHS-R antagonist increases plasma insulin concentrations in gastrectomized and normal rats similarly. (2) The ghrelin level is higher in the pancreatic vein than in the artery, indicative of release of ghrelin from pancreas. (3) mRNAs and proteins for ghrelin and GHS-R are expressed in pancreatic islets. (4)

Glucose-induced insulin release from perfused pancreas and isolated islets are augmented by GHS-R antagonists and ghrelin immunoneutralization. (5) Glucose-induced insulin release from isolated islets of Ghr-KO mice was greater than that of wild-type mice. (6) Ghrelin directly acts on islet β -cells to inhibit glucose-induced $[Ca^{2+}]_i$ increase and insulin release. Thus, pharmacological, immunological, and genetic blockade of ghrelin or ghrelin action in pancreatic islets all markedly enhanced glucose-induced insulin release (Fig. 3). These findings suggest that ghrelin is produced and released from pancreatic islet cells and act on islet β -cells via autocrine and/or paracrine manner, thereby suppressing glucose-induced insulin release (Fig. 3).

It is of particular importance to clarify how ghrelin and its receptor in islets are regulated under physiological and pathological conditions, including fast/fed, lean/obese and normoglycemic/diabetic states. Precise and relative roles of islet-derived and stomach-derived ghrelin in multiple steps of glucose metabolism remain to be further clarified. Although ghrelin employs unique molecules of $G\alpha_{12}$ and K_v , its signaling mechanisms in islet β -cells remain to be further elucidated. Chronic effects of the ghrelin system and its suppression on islet β -cells and glucose metabolism require further studies.

ACKNOWLEDGEMENTS

We thank Dr. Kangawa and Dr. Hosoda for providing ghrelin and ghrelin antiserum and for valuable discussion, and Dr. Kojima for providing ghrelin knockout mouse and for valuable discussion. We also thank Ms. Warashina, Ms. Ookuma and Ms. Motoshima for technical assistance, and Ms. Sakamoto for secretarial assistance. This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (TY, KD, MN, MK), those on Priority Areas (15081101) (TY) and (19045026) (KD), an Insulin Research Award from Novo Nordisk

Pharma Ltd. (TY), and grants from the 21st century Center of Excellence (COE) program (TY), Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan (TY), Japan Diabetes Foundation (TY) and Smoking Research Foundation (TY).

REFERENCES

- [1] Kojima M, Hosoda H, Date Y, *et al.* Ghrelin is a growth-hormone-releasing peptide from stomach. *Nature* 1999; 402: 656-60.
- [2] Howard AD, Feighner SD, Cully DF, *et al.* A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; 273: 974-7.
- [3] Ariyasu H, Takaya K, Tagami T, *et al.* Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; 86: 4753-8.
- [4] Nakazato M, Murakami N, Date Y, *et al.* A role for ghrelin in the central regulation of feeding. *Nature* 2001; 409: 194-8.
- [5] Kojima M, Kangawa K. Drug insight: the functions of ghrelin and its potential as a multitargeted hormone. *Nat Clin Pract Endocrinol Metab* 2006; 2: 80-8.
- [6] Tschop M, Weyer C, Tataranni PA, *et al.* Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; 50: 707-9.
- [7] Ariyasu H, Takaya K, Hosoda H, *et al.* Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* 2002; 143: 3341-50.
- [8] Shiiya T, Nakazato M, Mizuta M, *et al.* Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; 87: 240-4.
- [9] Ikezaki A, Hosoda H, Ito K, *et al.* Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes* 2002; 51: 3408-11.
- [10] Poykko SM, Kellokoski E, Horkko S, *et al.* Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. *Diabetes* 2003; 52: 2546-53.
- [11] Broglio F, Benso A, Castiglioni C, *et al.* The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. *J Clin Endocrinol Metab* 2003; 88: 1537-42.
- [12] Broglio F, Arvat E, Benso A, *et al.* Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 2001; 86: 5083-6.
- [13] Broglio F, Arvat E, Benso A, *et al.* Endocrine activities of cortistatin-14 and its interaction with GHRH and ghrelin in humans. *J Clin Endocrinol Metab* 2002; 87: 3783-90.
- [14] Dezaki K, Hosoda H, Kakei M, *et al.* Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in β -cells: implication in the glycemic control in rodents. *Diabetes* 2004; 53: 3142-51.
- [15] Dezaki K, Kakei M, Yada T. Ghrelin employs $G\alpha_2$ and activates Kv channels to attenuate glucose-induced Ca^{2+} signaling and insulin release in islet β -cells: Novel signal transduction of ghrelin. *Diabetes* 2007; 56: 2319-27.
- [16] Dezaki K, Sone H, Koizumi M, *et al.* Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 2006; 55: 3486-93.
- [17] Sun Y, Asnicar M, Saha PK, *et al.* Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metab* 2006; 3: 379-86.
- [18] Bednarek MA, Feighner SD, Pong SS, *et al.* Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J Med Chem* 2000; 43: 4370-6.
- [19] Shiao YS, Huang PT, Liou HH, *et al.* Structural basis of binding and inhibition of novel tarantula toxins in mammalian voltage-dependent potassium channels. *Chem Res Toxicol* 2003; 16: 1217-25.
- [20] Asakawa A, Inui A, Kaga T, *et al.* Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003; 52: 947-52.
- [21] Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol* 2003; 23: 7973-81.
- [22] Wortley KE, Anderson KD, Garcia K, *et al.* Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. *Proc Natl Acad Sci USA* 2004; 101: 8227-32.
- [23] Wortley KE, del Rincon JP, Murray JD, *et al.* Absence of ghrelin protects against early-onset obesity. *J Clin Invest* 2005; 115: 3573-8.
- [24] De Smet B, Depoortere I, Moechars D, *et al.* Energy homeostasis and gastric emptying in ghrelin knockout mice. *J Pharmacol Exp Ther* 2006; 316: 431-9.
- [25] Gauna C, Delhanty PJ, Hofland LJ, *et al.* Ghrelin stimulates, whereas desoctanoyl ghrelin inhibits, glucose output by primary hepatocytes. *J Clin Endocrinol Metab* 2005; 90: 1055-60.
- [26] Heijboer AC, van den Hoek AM, Parlevliet ET, *et al.* Ghrelin differentially affects hepatic and peripheral insulin sensitivity in mice. *Diabetologia* 2006; 49: 732-8.
- [27] Date Y, Nakazato M, Hashiguchi S, *et al.* Ghrelin is present in pancreatic α -cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; 51: 124-9.
- [28] Volante M, Allia E, Gugliotta P, *et al.* Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J Clin Endocrinol Metab* 2002; 87: 1300-8.
- [29] Wierup N, Svensson H, Mulder H, *et al.* The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002; 107: 63-9.
- [30] Wierup N, Yang S, McEvelly RJ, *et al.* Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. *J Histochem Cytochem* 2004; 52: 301-10.
- [31] Wierup N, Sundler F. Ultrastructure of islet ghrelin cells in the human fetus. *Cell Tissue Res* 2005; 319: 423-8.
- [32] Prado CL, Pugh-Bernard AE, Elghazi L, *et al.* Ghrelin cells replace insulin-producing β cells in two mouse models of pancreas development. *Proc Natl Acad Sci USA* 2004; 101: 2924-9.
- [33] Doi A, Shono T, Nishi M, *et al.* IA-2 β , but not IA-2, is induced by ghrelin and inhibits glucose-stimulated insulin secretion. *Proc Natl Acad Sci USA* 2006; 103: 885-90.
- [34] Zhang CY, Baffy G, Perret P, *et al.* Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. *Cell* 2001; 105: 745-55.
- [35] Parton LE, Ye CP, Coppari R, *et al.* Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature* 2007; 449: 228-32.
- [36] Kohno D, Nakata M, Maekawa F, *et al.* Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway. *Endocrinology* 2007; 148: 2251-63.
- [37] Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; 407: 908-13.